THE NATURE OF THE BACTEROIDS OF THE LEGUMINOUS NODULE AND THE CULTURE OF *RHIZOBIUM LEGUMINOSARUM.*

By R. Greig Smith, M.Sc., Macleay Bacteriologist to the Society.

In a recent publication* L. Hiltner discusses the views of Stutzer with regard to the nature of the bacteroids of the nodules of the *Leguminosæ*. The generally accepted view is that the bacteroids of the nodule are degenerate or involution forms of *Rhizobium leguminosarum*. Stutzer considers that they represent a higher and not a lower type of growth, and to this Hiltner replies that he cannot see why they should be so considered. His own view is that the bacteroids are simply enlarged bacteria.

In a former paper† I showed that the branching forms were really simple cells contained in a branching capsule. This is the case both with the organisms in artificial culture and in the root nodules. One has only to extract the colouring matter from the capsule after staining a bacterial film to see the simple cells within. There is no reason to suppose that the γ or the Y form is either degenerate or specialised; they are simply single cells contained in the bulky mother capsule from which the daughter cells are unable to escape until they have increased in size and become stronger or until the capsular envelope has become dissolved either partially or wholly by the fluids of the plant tissue.

---

* Hiltner, Centralblatt für Bakt. ii. Abt. vi. 273.

† These Proceedings, 1899, Part 4, pp. 653-673.

We have to remember that Prazmowski saw that the infecting thread contained rod-like cells, and that Maria Dawson while corroborating this noted that the gelatinous membrane (capsule) of the tube either became dissolved, whereby the rods were liberated, or the cells budded off *Dematium*-like. There is no reason to suppose that the same thing does not occur with the bacteroids; it is as unnecessary to suppose that the branching infecting thread is of a nature different to the branching bacteroids as that the cells in a chain of bacilli differ from a single isolated cell of the same species. The γ form of *Rhizobium* differs from the Y form only in the age of the daughter cells. In the γ form one or both of the daughter cells are immature (buds), while in the Y form they are mature (rods) and ready to escape from the enclosing membrane. There should never have been any question of degeneration or of specialisation: they are simply normal cells. Beijerinck has shown that the nature of the capsule can be altered at will in artificial media, and that forms precisely similar to those seen in the nodule can be obtained in artificial culture. The condition necessary for this appears to be the presence in the faintly acid culture fluid of potassium phosphate and a thin layer of fluid in the culture flasks.

The γ and Y bacteroidal forms are always observed in the young cultures in artificial media: in old cultures only the rod forms are present. This is the case not only with the nodule-former, but also, according to Sewerin and corroborated by the writer, with *Mycobacterium denitrificans*, an organism that produces similar branching forms in young (24 hours) cultures. This is in itself enough to negative the idea that these are degeneration forms, and, as I have pointed out, the specialisation hypothesis is unnecessary.

As for Hiltner's view that the bacteroids are large bacteria, it is quite possible, but it is not always so, as in working with the lupin bacteroids I have seen little difference in size between the cells contained within the bulky capsule and the cells obtained in artificial culture. Bacteria undoubtedly differ in size according to the media in which they are cultivated, and, as I have

shown, *Bac. megatherium* occurs in the acid fluid of the nodules similar in size to that originally observed by De Bary.

In a Scandinavian publication abstracted in the Experimental Station Record, xi, 1013, L. Hiltner, after summarisiug previous investigations relative to the fixation of atmospheric nitrogen, says that the nodule organisms are true parasites and secrete peculiar substances that cause the root hairs to shrivel up. "The injurious influence of the secretory products disappears when the tubercles attain their final development, but since these products continue to form inside the mature tubercle the supposition is that they are immediately converted into substances harmless to the plant. Such a conversion takes place with the co-operation of the host plant by supplying the organism with a part of the nutritive substances produced by the plant. This is further corroborated by the fact that from legumes and alders bacteria can be grown only in nutrient media containing extracts from the roots of leguminous or alder plants. The exclusive preference which is shown by *Bacillus radicicola* to leguminous plants tends to prove that the *Leguminosæ* alone are capable of producing the substances necessary for bacteria, the nature of which is being investigated." Although infusions of leguminous plants are commonly employed for the culture of the organisms that frequent the root nodules, it must not be assumed from this that the plant extract is absolutely necessary for the growth. The bacteriologist employs media which he considers will be best suited to the growth of the particular organism. For this reason extract of meat is used for the bacteria that are parasitic in animals, whey is used for milk bacteria, beer or yeast extract for the saccharomycetes, and so on. But most of the bacteria parasitic in animals will grow in media devoid of meat extract, and milk bacteria in media containing no milk. It is true that the nodule-formers are not found in plants other than the *Leguminosæ*, but we are no more entitled to assume from this that leguminous plants contain substances absolutely necessary for the growth of *Rhizobium* than that the tissues of man alone contain substances that are absolutely necessary for the growth of *Bact. typhi*, *Vibrio*

cholerae, &c. In fact, as I pointed out in the paper already quoted, "Extract of lupins or other leguminous plant does not seem a necessity for the culture media. Grass will do quite as well, and for that matter the plant extract might be left out entirely." Fairly luxuriant cultures were obtained upon a gelatine medium containing glucose and inorganic salts, and since that time I have cultivated the organism upon a medium containing faintly acid agar (2 %), glucose (2 %) and inorganic salts ($CaCl_2$ and KH_2PO_4) nearly neutralised with KOH.* On the latter medium there is no nitrogen except that which may be present as impurity in the washed agar, the glucose or the tap water. I have also grown the organism in an agar-free fluid medium prepared exactly as the agar medium. Such a fluid after inoculation becomes turbid and forms a slight sediment of organisms together with a bulky zoogloea cloud or sedimentary film.

The apparent growth in this very poor medium led to the belief that fixation of nitrogen might have occurred, but this was dispelled when the experimental flasks holding 250 c.c. of culture fluid were found to contain exactly the same amount of nitrogen (0·6 milligrm.) as was contained in control flasks.

* The method of preparing the faintly acid medium is described in these Proceedings, 1899, Part 4, p. 663.